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Ecological and Biogeochemical Interactions Constrain Planktonic Nitrogen Fixation in Estuaries

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Abstract

Many types of ecosystems have little or no N_2 fixation even when nitrogen (N) is strongly limiting to primary production. Estuaries generally fit this pattern. In contrast to lakes, where blooms of N_2-fixing cyanobacteria are often sufficient to alleviate N deficits relative to phosphorus (P) availability, planktonic N_2 fixation is unimportant in most N-limited estuaries. Heterocystic cyanobacteria capable of N_2 fixation are seldom observed in estuaries where the salinity exceeds 8–10 ppt, and blooms have never been reported in such estuaries in North America. However, we provided conditions in estuarine mesocosms (salinity over 27 ppt) that allowed heterocystic cyanobacteria to grow and fix N_2 when zooplankton populations were kept low. Grazing by macrozooplankton at population densities encountered in estuaries strongly suppressed cyanobacterial populations and N_2 fixation. The cyanobacteria grew more slowly than observed in fresh waters, at least in part due to the inhibitory effect of sulfate (SO_4^{2-}), and this slow rate of growth increased their vulnerability to grazing. We conclude that interactions between physiological (bottom–up) factors that slow the growth rate of cyanobacteria and ecological (top–down) factors such as grazing are likely to be important regulators excluding planktonic N_2 fixation from most Temperate Zone estuaries.

Key words: nitrogen fixation; estuaries; eutrophication; nitrogen limitation; grazing; heterocystic cyanobacteria.

Introduction

Nitrogen (N) availability limits productivity across broad classes of terrestrial and aquatic ecosystems, including most temperate forests, estuaries, and grasslands. In many other ecosystems, such as most tropical forests and temperate lakes, N_2 fixation is often a significant driver, bringing the N supply into stoichiometric balance with other nutrients, so that N is seldom limiting (Vitousek and Howarth 1991). Why N limitation persists in some ecosystem types despite the widespread biological capability to fix N_2 is a major paradox. The ecological controls on N_2 fixation are poorly understood (Vitousek and others 2002), yet they are critical to predicting the consequences of human-driven acceleration of the N cycle.

Temperate Zone lakes and estuaries provide an important contrast for understanding the potential for N_2 fixation to alleviate N limitation (Vitousek and Howarth 1991; Carpenter and others 1998). Many of these ecosystems are productive due to nutrient inputs from human disturbance in the landscape. In many such temperate lakes, annual production is controlled by phosphorus (P), in part because N_2 fixation by heterocystic cyanobacteria can alleviate ecosystem deficits in

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Table 1. Mean Calanoid Copepod Densities and Inorganic Nutrients Characterizing the Mesocosms

Experiment	Treatment	Zooplankton (Individuals L^{-1})	DIN (μM)	DIN:DIP (M)
1996	HGr	46 ± 12	0.98 ± 0.13	0.22 ± 0.05
	LGr	1.1 ± 0.2	1.3 ± 0.36	0.30 ± 0.06
1998	HGr	44 ± 3	0.36 ± 0.03	0.14 ± 0.03
	LGr	0.5 ± 0.1	0.58 ± 0.10	0.97 ± 0.37

HGr, high grazing; LGr, low grazing
Weekly means ± 1 standard error; n = 10 (1996), n = 7 (1998).
Zooplankton densities include adults, copepedites, and most nauplii.

the availability of N relative to P (Schindler 1977). However, many productive estuaries that are persistently and strongly N limited nonetheless have undetectable rates of planktonic N_2 fixation (Howarth and others 1988; Paerl 1996). Although N_2-fixing cyanobacteria rarely occur in the plankton of most estuaries, several taxa of heterocystic cyanobacteria have the capacity to grow and fix N_2 across a wide range of environmental conditions, including salinities that span the range found in estuaries (Carr and Whitton 1982; Paerl and Zehr 2000). Several single-factor hypotheses have been proposed to explain the relative absence of planktonic N_2 fixation in estuaries (Howarth and others 1988; Smith 1990; Paerl 1996; NRC 2000), but none have proven adequate. A recent model suggested that differential sensitivity of developing heterocystic cyanobacteria populations in the plankton to grazing, combined with slower growth rates under saline estuarine conditions, can potentially suppress blooms over a seasonal time frame (Howarth and others 1999).

Understanding the controls on planktonic N_2 fixation in estuaries is critical to addressing the rapidly growing problem of coastal eutrophication. Nitrogen is now the largest pollution problem in the coastal waters of the United States, and two-thirds of the coastal rivers and bays in the United States are moderately to severely degraded as a result of nutrient enrichment (NRC 2000). Some workers have suggested that there is little value in controlling N inputs to estuaries, since it only stimulates cyanobacteria blooms and increases rates of N_2 fixation, as commonly occurs in nutrient-enriched lakes (Hecky 1998; Tyrell 1999). However, if N_2 fixation in estuaries is constrained by other factors, such as those we study here, reducing N inputs to estuaries will improve water quality.

Methods

We ran two mesocosm experiments during the summers of 1996 and 1998 using water from Narragansett Bay, Rhode Island, a high-salinity estuary (27–32 ppt) in which primary production is strongly N limited (Oviatt and others 1995; NRC 2000). The mesocosms were 1.83-m diameter, 1.15 m-deep (3-m^3) tanks constructed of Kalwal fiberglass and covered with opaque insulation. The tanks were open to the atmosphere; bubblers prevented stratification and provided turbulence within the range estimated for Narragansett Bay and other estuaries (Howarth and others 1993; Marino 2001). Tanks were filled over three to four tidal cycles at the beginning of each experiment. Experiments were run in batch mode, with no further introduction of water during the experiment except for small amounts added with a weekly cyanobacterial seeding source (Marino 2001) and water from precipitation. All tanks were enriched with P twice per week at a loading rate of 160 μmol m^{-3} d^{-1} to yield a dissolved molar N:P ratio well below the Redfield ratio (16:1); no N was added other than that occurring in precipitation.

Macrozooplankton were kept at low abundance in one subset of the mesocosms (low grazing = "LGr") by adding zooplanktivorous fish (15 juvenile *Menidia beryllina* per tank; ~0.5 g fish biomass). Abundances more characteristic of temperate estuaries were maintained in other mesocosms that had no fish (high grazing = "HGr") (Table 1). Mean zooplankton densities (predominantly *Acartia tonsa*) in HGr mesocosms were within the seasonal range observed in Narragansett Bay (Durbin and Durbin 1981). Both experiments included other treatments not presented here; the grazing manipulation was the most significant in both years (Marino 2001). Four replicate tanks of each grazing level were run in 1996 and eight were run in 1998, for 70 and 59

days, respectively. The study of phytoplankton dynamics was ended 2 weeks early in 1998 due to an unexplained increase of zooplankton in some LGr mesocosms.

Total and dissolved inorganic nutrients were measured weekly on integrated water column samples and on an event basis in bulk precipitation using standard techniques (Grasshoff and others 1983; Marino 2001). Particulate N was measured on pooled freeze-dried samples taken in each mesocosm over the duration of the 1998 experiment, using a Carlo-Erba CN analyzer (Leica Microsystems, Wetzlar, Germany). These data were used in the calculation of total N increases. Phytoplankton were sampled weekly throughout both experiments and daily for approximately 2-week periods in both years. Samples were preserved with Lugol's solution and counted for cyanobacterial cells and heterocysts on a Wild M-40 inverted microscope after gravitational settling. Macrozooplankton were sampled through the water column on a weekly basis using 73- or 100-μ nets to filter 13–65 L of water (capture efficiency of adults plus copepedites and most nauplii more than 99% for both) (Chan 2001).

Heterocystic cyanobacteria response to grazing was described using mean density as the derived variable; cell and heterocyst densities were log-transformed before statistical analysis to equalize variance both within and across treatments and to weight data properly over time. Time-weighted means of the transformed data were analyzed by analysis of variance (ANOVA) using a statistical package for desktop computers (Stat View; SAS Institute, Cary, NC, USA).

Results and Discussion

Even though N_2-fixing cyanobacteria have not been observed in the plankton of Narragansett Bay (Smayda 1973), we were able to provide conditions where filamentous cyanobacteria with heterocysts (primarily *Anabaena* sp.) grew for 1–2 months. The demonstration that planktonic, heterocystic cyanobacteria can grow and fix N_2 at high salinity is in itself significant, because these organisms have not been observed in the plankton of the saline regions (more than 10–15 ppt) of any North American estuaries and have only been found in a very few estuaries worldwide. Zooplankton had a strong negative influence on the abundance of both cyanobacterial cells and heterocysts (the active site of N_2 fixation) (Figure 1), as well as the total N increase attributable to N_2 fixation during the more detailed 1998 experiment (0.18 versus 0.42 N in HGr and LGr, respectively; $P = 0.0002$) (Marino 2001). We believe that the effect of zooplankton on cyanobacteria abundance was direct (that is, filament clipping and/or consumption), rather than an indirect result of differential recycling of N and P as has been observed in some lake experiments (Sterner and others 1992). We found no significant treatment differences in dissolved inorganic nitrogen (DIN) over time, and molar DIN:DIP ratios were consistently favorable for N_2 fixation in all mesocosms (less than 4:1) (Table 1). Concurrent short-term grazing experiments confirmed that the dominant zooplankter in our mesocosms (*Acartia tonsa*) readily consumed cyanobacterial cells (Chan 2001).

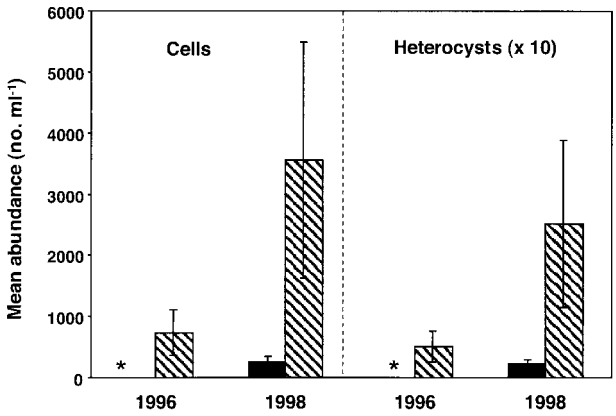

Figure 1. Effect of macrozooplankton grazing on mean abundance of *Anabaena* heterocysts and cells in seawater mesocosms. Striped bars indicate low zooplankton biomass treatment (LGr); solid bars indicate the high zooplankton biomass (HGr) treatment; asterisk (*) indicates that no cells or heterocysts were detected at any time. Cell and heterocyst values are time-weighted treatment means during the period in which *Anabaena* were observed (12 days in 1996 and 21 days in 1998); error bars are ± 1 standard error. Treatment differences within years are significant at $P \leq 0.05$ for heterocyst and cell abundances. Note scale change for heterocyst data.

N_2-fixing heterocystic cyanobacteria may be particularly sensitive to grazing, as a threshold number of photosynthetic cells (15–35 in our experiments) is needed in a filament to provide the substantial energy needs of heterocyst differentiation and N_2 fixation (Howarth and others 1999; Chan 2001). Grazing can reduce cyanobacterial filament length (Burns and Xu 1990; Schaffner and others 1994; Chan 2001), and greatly influenced this size-structure dependent aspect of cyanobacterial growth on N_2 in our experiments. *Anabaena* present in the HGr treatments exhibited a 67% reduction in filament length and a 61% reduction in heterocyst frequency per filament relative to that of *Anabaena*

Table 2. Effects of Grazing on Cyanobacterial Morphology in Mesocosm Experiments

Experiment	Treatment	Mean Filament Length (Cells)	Heterocyst Frequency (Filament^{-1})	% of Filaments Too Short to Support One Heterocyst
1996	HGr	No filaments	No filaments	No filaments
	LGr	42.1 ± 12.1	2.9 ± 0.7	Not estimated
1998	HGr	15.4 ± 1.6	1.4 ± 0.1	23.2 ± 2.5
	LGr	46.3 ± 2.1	3.6 ± 0.3	11.2 ± 1.6

HGr, high grazing; LGr, low grazing
Mean ± 1 standard error.
Differences within years are significant at $P < 0.0005$.

populations in the LGr mesocosms (Table 2). Cyanobacteria actively fixing N_2 in freshwater experiments have been observed to grow faster when filaments were longer (Chan 2001).

Although planktonic N_2-fixing cyanobacteria with heterocysts were present in our mesocosms, and the removal of grazing significantly enhanced their numbers, the population in the LGr treatment (1998 data) (Figure 1) was 35-fold less than that observed in a comparable freshwater mesocosm experiment with identical nutrient loading and similar reductions in macrozooplankton grazing (Howarth and others 1993). Also, in a long-term whole-lake experiment with natural zooplankton populations and very similar P loading and N:P ratios as in our mesocosms, mean seasonal heterocyst abundances were 100- to 300-fold greater than in our zooplankton (HGr) treatments (Findlay and others 1994; Marino 2001). Seasonal input of N from N_2 fixation in both these freshwater experiments was large enough to result in P-limited systems (Howarth and others 1993; Findlay and others 1994). In striking contrast, our estuarine mesocosms remained N limited throughout the growing season, based on N:P ratios (NRC 2000), regardless of the intensity of grazing pressure.

The difference in cyanobacterial response between our estuarine experiments and those in fresh water suggests intrinsically slower growth of N_2-fixing cyanobacteria in saline waters. A literature compilation of maximum growth rates of heterocystic cyanobacteria (*Anabaena*, *Aphanizomenon*, and *Nodularia* spp.) supports this idea; growth averaged three to four times higher under freshwater conditions than under estuarine salinity conditions (Figure 2). We believe that this slower growth is due, at least in part, to an inhibitory effect of SO_4^{2-} on cyanobacterial growth in saline waters. One mechanism for a negative effect of SO_4^{2-} on N_2-fixing cyanobacteria is an inhibition of molybdenum (Mo) uptake at higher SO_4^{2-} levels (Howarth and Cole 1985). Previous studies have demonstrated an inhibitory SO_4^{2-} effect on Mo uptake by a variety of organisms growing on N sources that require Mo-

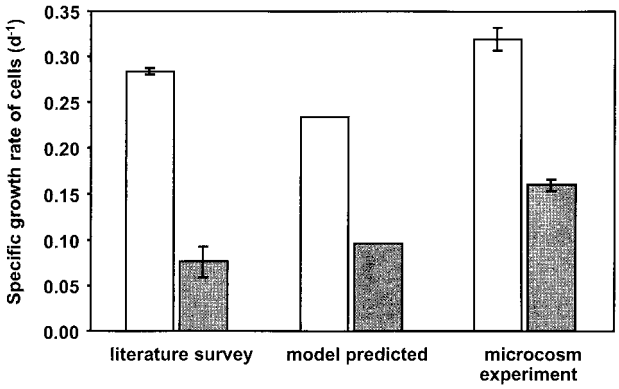

Figure 2. Comparisons of heterocystic cyanobacteria growth under freshwater (*white bar*) and estuarine (*solid bar*) SO_4^{2-} and Mo concentrations. Literature data are from cultures grown under freshwater (n = 38) and estuarine (n = 27) conditions; reported maximum growth rates were normalized to 20°C using a Q_{10} of 2 (Chan 2001). Model-predicted growth rates are from Howarth and others (1999). Freshwater maximum rate is set based on the literature. Estuarine/seawater growth rate is calculated by scaling freshwater value using a competitive inhibition kinetic model of Mo uptake by SO_4^{2-} at $[SO_4^{2-}]$ = 28 mM and [Mo] = 0.1 μM. Maximum rates are converted to specific growth rates using the Monod equation (assuming P-enriched ambient conditions of 1 μM DIP and a P half-saturation coefficient of 2 μM). Microcosm experiment data are from Marino (2001). Freshwater value represents growth of N_2-fixing *Anabaena* spp. under ambient pond SO_4^{2-} and Mo, moderate light, DIP maintained at 1 μM, and $NaHCO_3$ added to buffer pH and guard against CO_2 limitation. Saline estuarine rate is from microcosms that also had SO_4^{2-} and Mo added to 28 mM and 0.1 μM, respectively. All data shown are means ± 1 standard error.

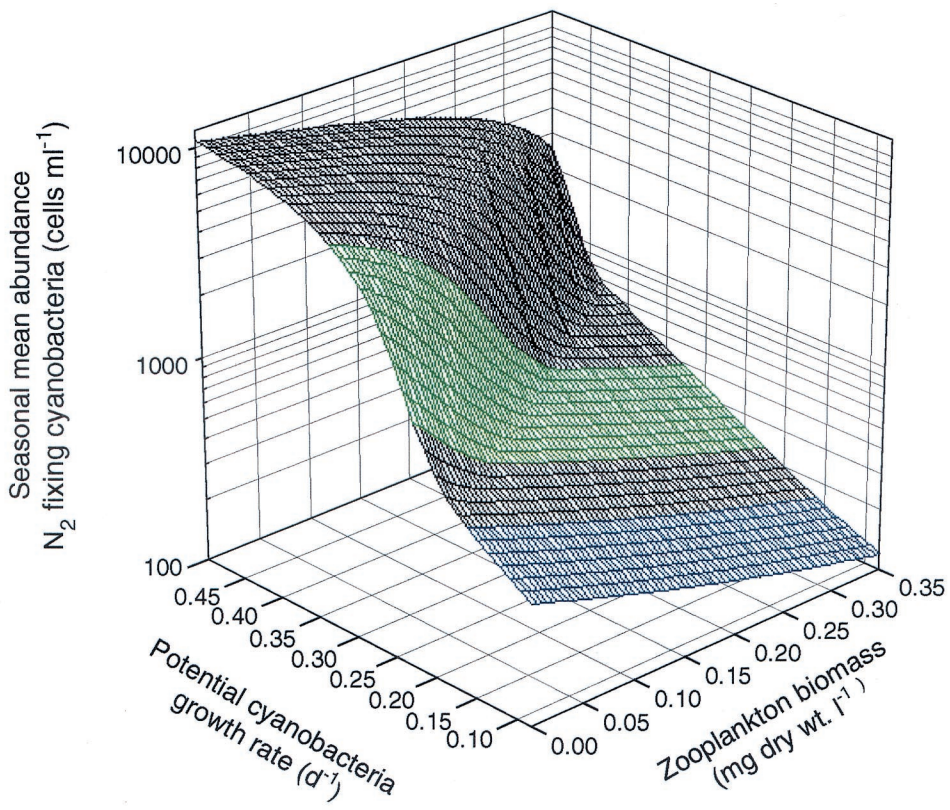

Figure 3. Modeled sensitivity of heterocystic cyanobacteria abundance to interacting controls by growth rate and zooplankton grazing for freshwater (green shading) and estuarine systems (blue shading). The model structure is presented elsewhere (Howarth and others 1999). Actual magnitude of seasonal mean abundance is sensitive to initial conditions of DIP, DIN, and cyanobacterial cell numbers for a given model run (1 μM, 10 μM, and 240 cells ml^{-1}, respectively here); however, the pronounced nonlinearity of the response surface is unaffected.

containing enzymes (N_2, NO_3^-), including eukaryotic phytoplankton, heterotrophic bacteria, and cyanobacteria (Cole and others 1986, 1993; Corcuera and others 1993). Sulfate has also been shown to inhibit N_2 fixation (Howarth and Cole 1985; Stal and others 1999) and to inhibit diatom growth on NO_3^- but not NH_4^+, where there is no Mo requirement (Cole and others 1986; Saros and Fritz 2000). In an 8-day microcosm experiment using water from a P-enriched freshwater pond with abundant N_2-fixing *Anabaena* spp., we found that the addition of SO_4^{2-} at seawater concentrations (28 mM) slowed both the growth and N_2 fixation rates of the cyanobacteria by two- to three-fold, as compared to microcosms with chloride salts added to control for ionic or osmotic effects or to those with no salt additions (Marino 2001). This result is consistent with the literature growth rate data and with previous model predictions (Figure 2).

To examine the interaction of the physiological growth constraint imposed by SO_4^{2-} with the ecological constraint imposed by zooplankton grazing in estuaries, we reran our model of N_2 fixation (Howarth and others 1999) using our new experimental and literature survey data (Figure 3). The underlying hypothesis does not rely on a systematic difference in grazing pressure across freshwater and estuarine systems, but rather on macrozooplankton grazing levels typically found in both systems being more effective in suppressing the development of a filamentous cyanobacteria bloom as estuaries become more saline. The model indicates a sharp nonlinearity; grazing is a large constraint on the production of cyanobacterial biomass when growth rates are slow, but it is far less constraining under the higher growth rates found in freshwaters. This nonlinearity results primarily from strong energetic limitations on N_2 fixation when cyanobacterial filaments are short; longer filaments better support heterocysts and are capable of more rapid growth (Howarth and others 1999; Chan 2001). In freshwater and brackish (less than 8–10 ppt salinity) waters where heterocystic cyanobacteria can achieve high cell growth rates, macrozooplankton grazing on cyanobacteria can occur (Schaffner and others 1994; Koski and others 2002), but it is often insufficient to control blooms (Carpenter and others 1995; Sellner 1997). However, under saline estuarine conditions where rates of cell growth are slower and heterocyst differentiation is thus constrained, such trophic control becomes effective in reducing bloom development under N-limited conditions.

We do not view the SO_4^{2-} effect as a single or

absolute constraint adequate to prevent nitrogenase synthesis and cell growth on N_2, but rather as a control on the gross growth rate of the heterocystic cyanobacteria and so the potential for a bloom to develop. Factors other than SO_4^{2-} may also differentially affect the growth of cyanobacteria in estuaries compared to lakes; thus, these factors would be expected to interact with grazing in a similarly nonlinear way (Figure 3). For example, iron (Fe) availability, which is thought to limit N_2 fixation in some oligotrophic ocean waters (Karl and others 2002), is likely quite different in lake and estuarine systems, in part because of the large differences in the solubility of Fe species in fresh water and seawater (Kester and others 1975). Other biogeochemical parameters such as DIP concentration and pH can affect the growth of cyanobacteria (Paerl 1996; Karl and others 2002). However, these factors are unlikely to lead to a systematic difference between estuaries and lakes, even though they may help to explain some of the variation in growth rates among systems with similar constraints on Mo and/or Fe availability (Howarth and others 1988).

Our results demonstrate the importance of considering the coupling of both bottom-up (physiological) and top-down (grazing) controls to understand how N_2 fixation can alleviate N limitation in lakes but not estuaries. The interaction of resource and trophic controls on primary productivity has been appreciated in lakes (Carpenter and others 2001) and oceanic systems (Price and others 1994). This study demonstrates that such interactions—rather than single or multiple physiological controls—can also regulate ecosystem N_2 fixation. Our analysis strongly suggests that an ecological amplification of a physiological-level constraint on growth, such as inhibition by SO_4^{2-}, is sufficient to exclude N_2-fixing cyanobacteria blooms from many estuaries. The influence of this mechanism in low-salinity nutrient-enriched systems such as much of the Baltic Sea is less clear (Elmgren 2001). The differential availability of nutrients essential for N_2 fixation (Fe, Mo, P) and the relative importance of any one of them in constraining growth rates of N_2-fixing organisms in terrestrial or oligotrophic oceanic systems may well differ from those in our study (Karl and others 2002; Vitousek and others 2002). Other ecological controls in addition to grazing, such as competition, may also be important in amplifying physiological constraints on N_2 fixation in some types of ecosystems (Vitousek and others 2002). Nonetheless, our results indicate that explicit consideration of interactions between physiological and ecological controls on biological N_2 fixation may be central to resolving the biogeochemical paradox of persistent N limitation of primary production in many of Earth's ecosystems.

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